

In the name of GOD

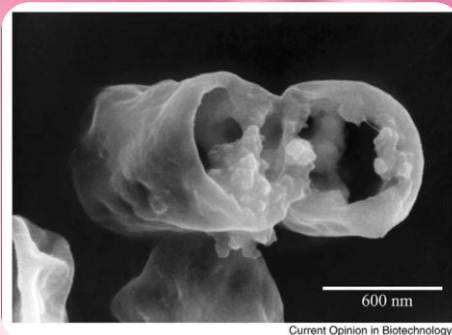
Journal Club & MSc Seminar



Bacterial ghosts

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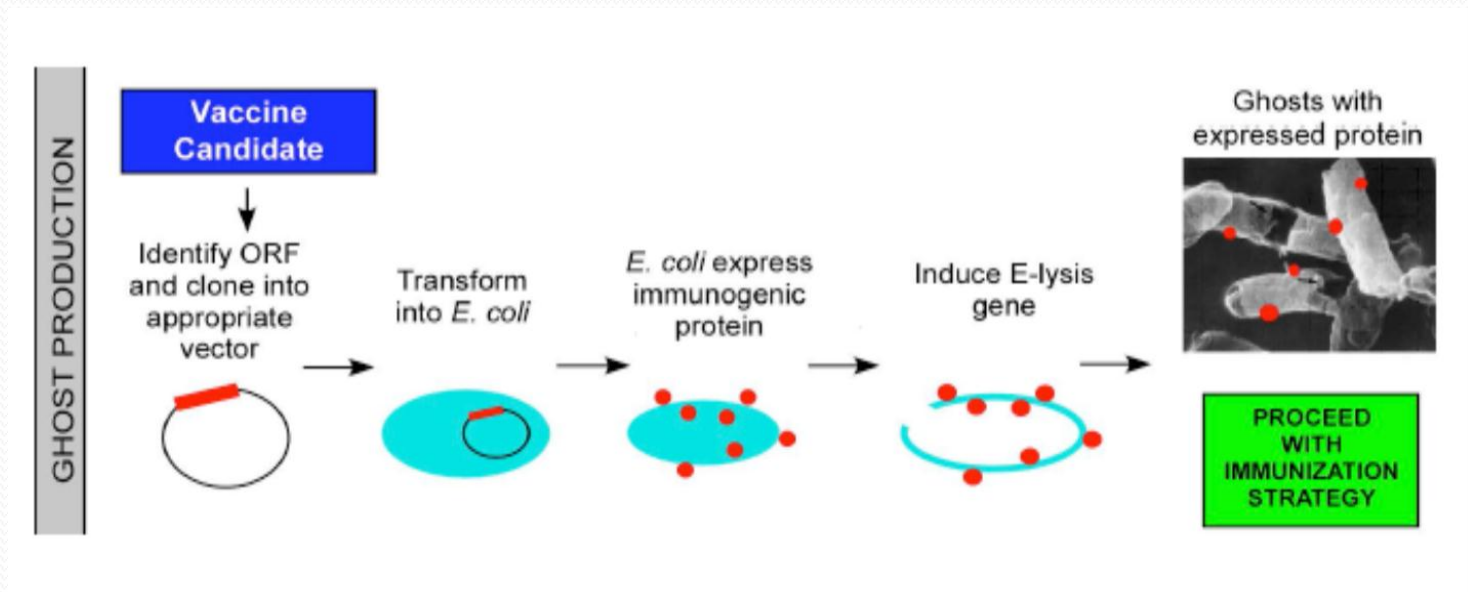
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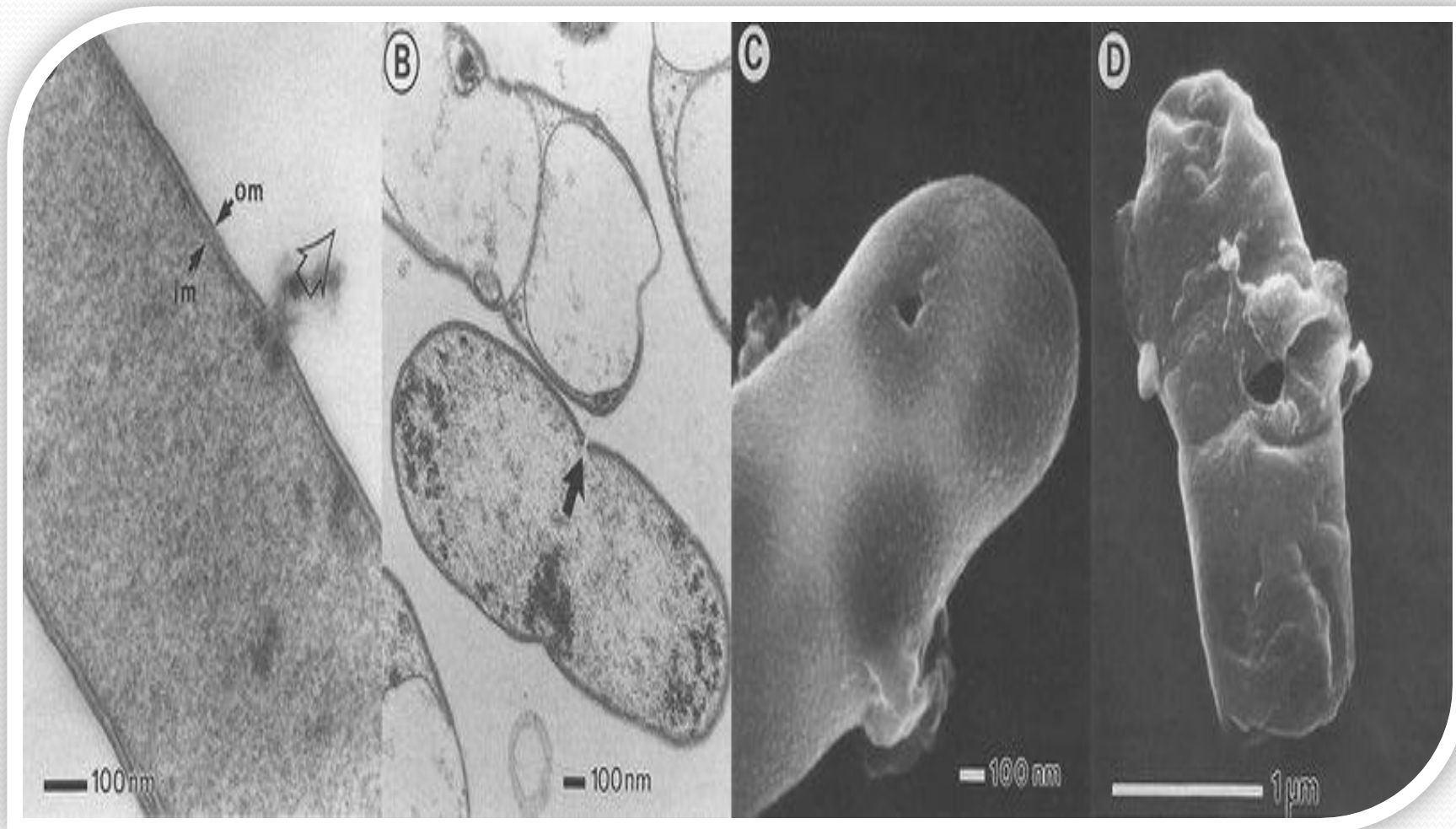
Bacterial Ghosts :



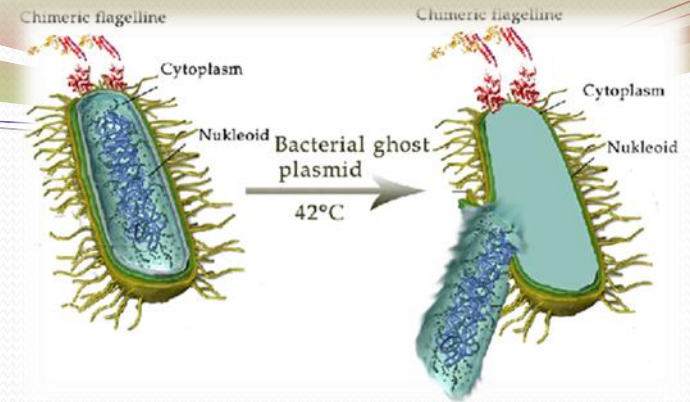
- The BGs is the:
 - bacterial **envelope** without its **internal content**.
 - with fully **intact surface** structures.
 - usually obtained from **Gram-negative bacteria** .
- Inducible expression of the **gene E** causes the fusion of the inner and outer membranes of the bacterial cells to form inter membrane tunnel through which all the cytoplasmic contents of the bacteria is expelled, whilst the inner and outer membrane structures are preserved and remain intact.

- BG are produced by expression of cloned gene E from **bacteriophage PhiX174** resulting cell lysis in Gram-negative bacteria.



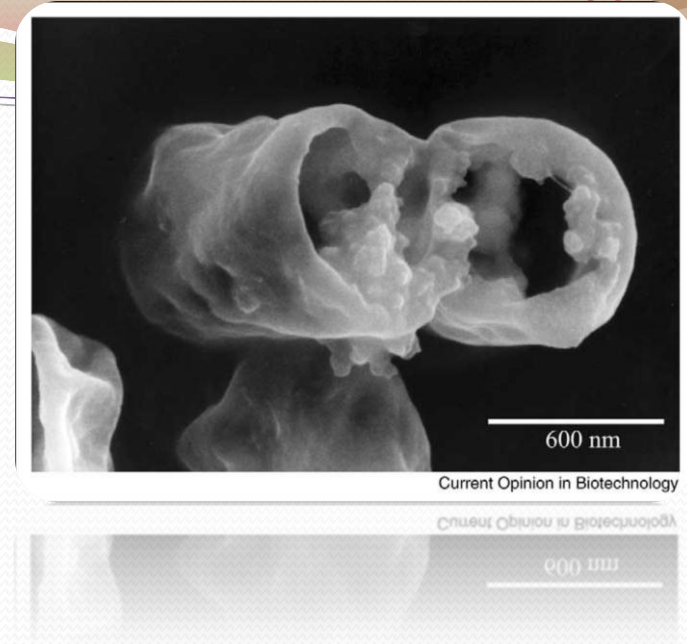


Bacterial Ghosts :

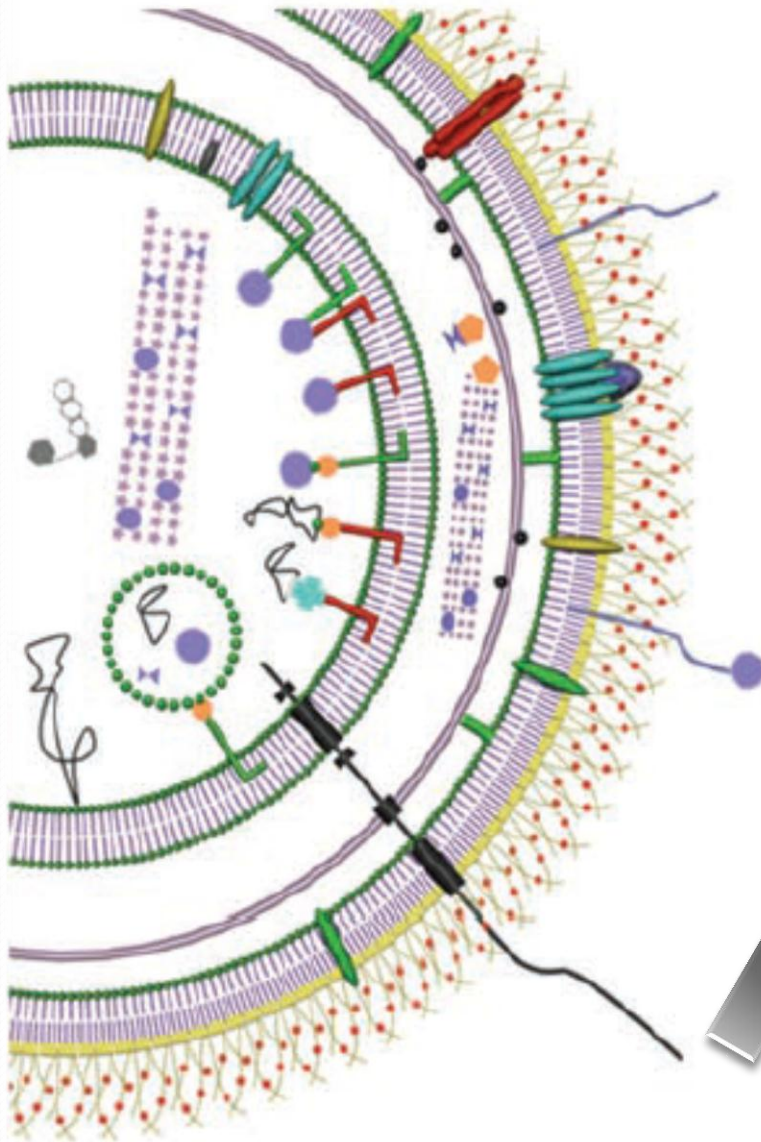


- The outer surface of BG contains all the appendices, :
 - pili
 - flagella
 - Lipopolysaccharide of the parent bacteria
- Their inner surface corresponds to the inside of the cytoplasmic membrane and its associated products .
- The space between both membranes is the periplasmic space which by its nature is a gel like environment rich in membrane derived oligosaccharides, specific enzymes, proteins and peptidoglycan.

Bacterial Ghosts :



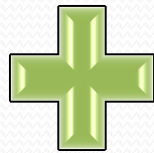
- They have a natural outer surface make-up which provides them with the original targeting functions of the bacteria they are derived from and are thus able to bind to and/or are **taken up** by specific cells or tissues of **animal, human** or **plant origin** .



Sector of BG
envelope

Bacterial Ghosts :

maintains all surface proteins of the original bacterium in its original state



possesses all the structural, immunogenic and bio adhesive properties of the original bacterium.



**mimic
the
living
bacteria**

Bacterial Ghosts are derived from:

❖ **BGs have been produced from various non-pathogenic, pathogenic and probiotic strains :**

- **Gram-negative bacteria**

- *Escherichia coli*
- *Klebsiella*
- *Salmonella*
- *Enterobacter*
- *Pseudomonas*
- *Vibrio*
- *Haemophilus*
- *Pasteurella* , *Bordetella*, *Helicobacter*, *Francisella*, *Brambamella*, ...

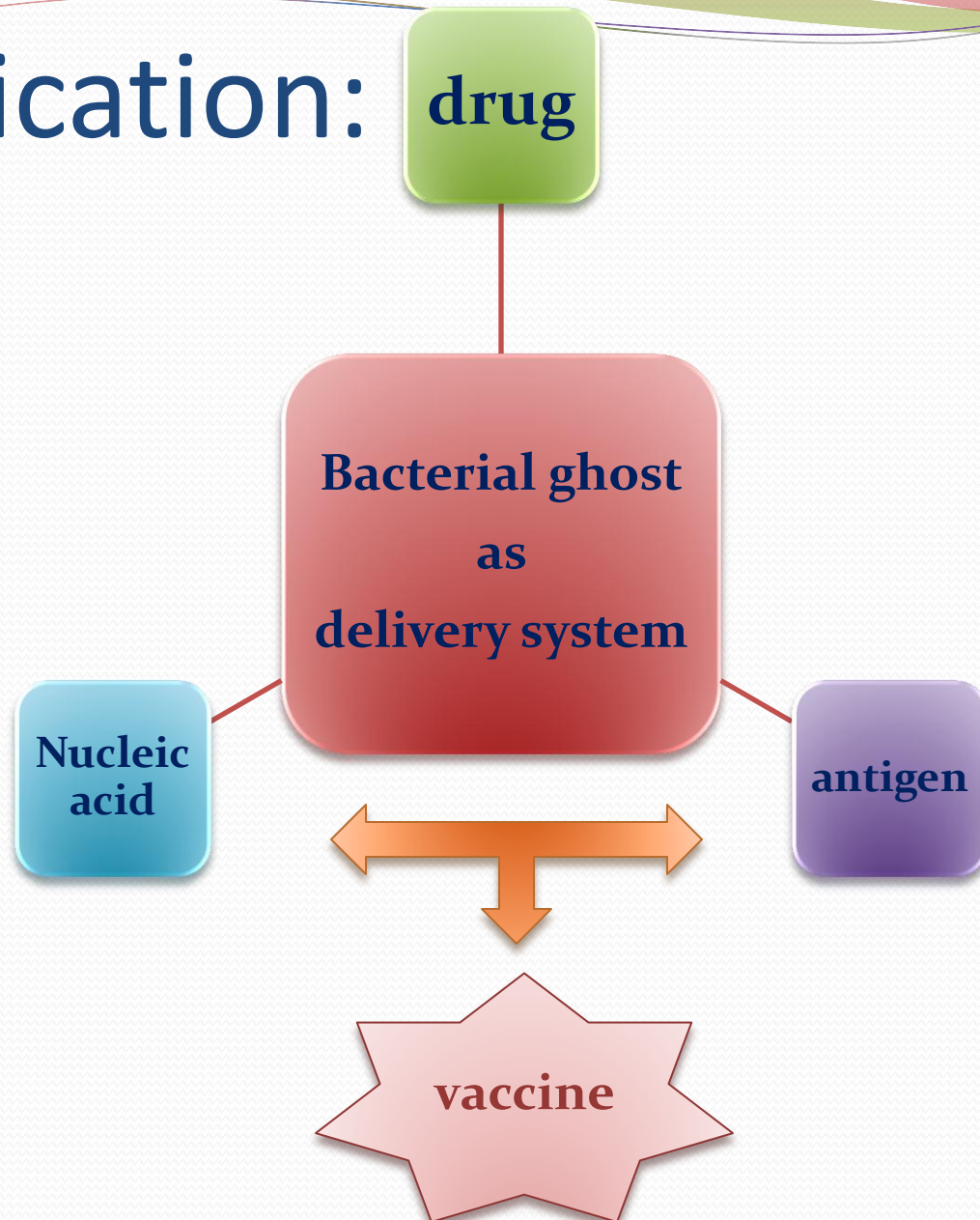
- **Gram-positive bacteria :**

- *Staphylococcus*, *Streptococcus* and *Bacillus*

Advantages of Bacterial Ghost:

- 1) Non-living carriers ➡ No risk of reversal to pathogenic form.
- 2) Ability to express recombinant proteins in a variety of locations on the Ghost.
- 3) long room temperature storage life after lyophilization.
- 4) low cost of production.
- 5) Strong adjuvant properties.
- 6) Good recognition and uptake by antigen-presenting cells
- 7) High loading capacity for DNA.
- 8) Targeting properties for different tissues.

Application:



Delivery systems for drug

- Recent investigations confirm the recognition of BGs by various types of tumor cells and the capacity of BGs to efficiently target and be internalized by melanoma, leukemia and colorectal carcinoma cells.
- PAMPs present on the surface of BGs help to increase targeting of tumor cells with BGs loaded with chemotherapeutic substances.
- A delivery system that transports chemotherapeutic drugs directly to the cytosol and nuclear area of target cells at levels sufficient to inhibit tumor cell proliferation would allow the use of decreased drug dosages, and thus lessen the negative impacts on people already challenged with serious diseases.



Contents lists available at ScienceDirect

Vaccine



❖ **Doxorubicin** (anti-neoplastic drug) loaded BGs made by simple resuspension and incubation of lyophilized BGs in Dox solution, were used as a model system for BGs and drug delivery.

❖ Delivery of Dox bound to the inner membrane of BGs increased its intracellular concentration within tumor cells (CaCo2) up to **42 times** compared to tumor cells incubated with an equivalent concentration of Dox solution.

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ABSTRACT

Bacterial ghosts (BGs) are empty bacterial envelopes of Gram-negative bacteria produced by controlled expression of cloned gene E, forming a lysis tunnel structure within the envelope of the living bacteria. BGs are devoid of cytoplasmic content and possess all bacterial bio-adhesive surface properties in their original state while not posing any infectious threat. BGs are ideally suited as an advanced drug delivery system (ADDS) for toxic substances in tumor therapy. The inner space of BGs can be loaded with either

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Delivery systems for antigens

- Compared with simple virus-like particle carriers, the bacterial cell offers **several compartments** for the deliver of immunogenic antigens and has a **greater capacity**.

Expression of an antigen

Flagellin
fimbriae
outer
membrane

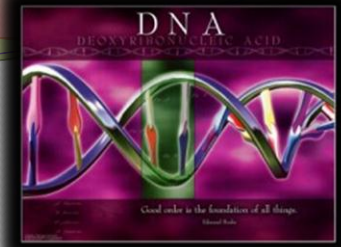
delivery system approaches has been reported in the development of a surface-display system based on the use of the **spore coat of *Bacillus subtilis***. This has an interesting advantage in that bacterial spores can survive extremes of **temperature, desiccation** and exposure to **solvents** and other **noxious chemicals**.

and impact on the
elicited immune
response

&

**better induction of
specific antibodies**

Delivery systems for DNA



- BGs have excellent DNA loading capacity varying from 4000 to 6000 plasmid copies per BG depending on the concentrations of DNA solution used .
- BGs loaded with plasmid DNA are efficiently **internalized and phagocytosed by both professional antigen-presenting cells (APCs) and tumor cells**. Cross-presentation of Ag delivered to DCs by BGs can activate both CD4+ and CD8+ T cells and stimulate the immune system to enhance the immune response against Ag expressed by target cells.

Bacterial ghosts as candidate vaccines



An ideal or practical vaccine should be:

1. Give life-long immunity .
2. Broadly protective against all variants of organism.
3. Prevent disease transmission.
4. Rapidly induce immunity.
5. Effective in all subjects (the old & very young).
6. Transmit maternal protection to the foetus.
7. Stable, cheap & safe.

Bacterial ghosts as candidate vaccines

- BG seem to fulfill the criteria mentioned above, as:
 - BG have an excellent safety profile as they are non-living vaccines which pose **no pathogenic threat or hazard of horizontal gene transfer** and do not require the addition of adjuvants.
 - BG are non-living bacterial envelope complexes with their surface components in their natural non-denatured arrangement.
 - BG can carry **additional antigens** (protein or DNA encoded) which are presented to the immune system as particles with intrinsic **adjuvant properties** addressing different toll-like receptors and inducing **humoral and cellular immune responses** towards the BG carrier and the target antigens.

Bacterial ghosts as candidate vaccines

- BG are **stable** in liquid suspension for several days and as freeze dried powder for several years.
- in addition to parenteral application routes BG can be administered in needle free form either by **oral aerogenic, intra-nasal, conjunctival, rectal or intravaginal** routes.
- there is a one step production process for plain BG vaccines or BG as carrier of subunit vaccine.

***Helicobacter pylori* vaccine development: Optimisation of strategies and importance of challenging strain and animal model**

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Abstract

Gastric infection with the Gram-negative bacterial pathogen *Helicobacter pylori* is widespread (approximately 50% of the human population is affected) and is associated with the induction of specific gastroduodenal disease. Although extensive studies in the *H. pylori* mouse model have demonstrated the feasibility of both therapeutic and prophylactic immunisations, the mechanism of vaccine-induced protection is still poorly understood. We report here on novel strategies to optimise the generation of *H. pylori* ghosts as vaccine candidates and highlight the need to concentrate on alternative animal models and the use of fully virulent *H. pylori* type I strains for vaccination. An effective vaccine strategy against *H. pylori* has the potential to significantly improve population health worldwide.

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Keywords: *H. pylori*; Bacterial ghosts; Vaccination; Gerbil model; Lysis gene cassette

Bacterial Ghosts as an Oral Vaccine: a Single Dose of *Escherichia coli* O157:H7 Bacterial Ghosts Protects Mice against Lethal Challenge

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Eugenij Bukin,³ Werner Lubitz,^{1,2} and Georgy Ignatyev³

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Enterohemorrhagic *Escherichia coli* (EHEC) is a bacterial pathogen that is associated with several life-threatening diseases for humans. The combination of protein E-mediated cell lysis to produce EHEC ghosts and staphylococcal nuclease A to degrade DNA was used for the development of an oral EHEC vaccine. The lack of genetic material in the oral EHEC bacterial-ghost vaccine abolished any hazard of horizontal gene transfer of resistance genes or pathogenic islands to resident gut flora. Intragastric immunization of mice with EHEC ghosts without the addition of any adjuvant induced cellular and humoral immunity. Immunized mice challenged at day 55 showed 86% protection against lethal challenge with a heterologous EHEC strain after single-dose oral immunization and 93.3% protection after one booster at day 28, whereas the controls showed 26.7% and 30% survival, respectively. These results indicate that it is possible to develop an efficacious single-dose oral EHEC bacterial-ghost vaccine.



RESEARCH

Open Access

Construction of a *Salmonella* Gallinarum ghost as a novel inactivated vaccine candidate and its protective efficacy against fowl typhoid in chickens

Atul A Chaudhari, Chetan V Jawale, Sam Woong Kim and John Hwa Lee*

Abstract

In order to develop a novel, safe and immunogenic fowl typhoid (FT) vaccine candidate, a *Salmonella* Gallinarum ghost with controlled expression of the bacteriophage PhiX174 lysis gene *E* was constructed using pMMP99 plasmid in this study. The formation of the *Salmonella* Gallinarum ghost with tunnel formation and loss of cytoplasmic contents was observed by scanning electron microscopy and transmission electron microscopy. No viable cells were detectable 24 h after the induction of gene *E* expression by an increase in temperature from 37 °C to 42 °C. The safety and protective efficacy of the *Salmonella* Gallinarum ghost vaccine was tested in chickens that were divided into four groups: group A (non-immunized control), group B (orally immunized), group C (subcutaneously immunized) and group D (intramuscularly immunized). The birds were immunized at day 7 of age. None of the immunized animals showed any adverse reactions such as abnormal behavior, mortality, or signs of FT such as anorexia, depression, or diarrhea. These birds were subsequently challenged with a virulent *Salmonella* Gallinarum strain at 3 weeks post-immunization (wpi). Significant protection against the virulent challenge was observed in all immunized groups based on mortality and post-mortem lesions compared to the non-immunized control group. In addition, immunization with the *Salmonella* Gallinarum ghosts induced significantly high systemic IgG response in all immunized groups. Among the groups, orally-vaccinated group B showed significantly higher levels of secreted IgA. A potent antigen-specific lymphocyte activation response along with significantly increased percentages of CD4⁺ and CD8⁺ T lymphocytes found in all immunized groups clearly indicate the induction of cellular immune responses. Overall, these findings suggest that the newly constructed *Salmonella* Gallinarum ghost appears to be a safe, highly immunogenic, and efficient non-living bacterial vaccine candidate that protects against FT.

populations among the PBMC of the immunized and non-immunized groups. Our data showed that all immunized groups showed significant lymphocyte activation responses upon antigen stimulation compared to the non-immunized groups. These lymphocyte activation responses clearly suggest that the lymphocytes (either B or T cells) from the immunized birds were activated upon antigen stimulation, possibly due to circulating memory cells in the peripheral blood of the immunized birds. These findings may indicate that the *Salmonella*

Taken together, these findings suggest that the newly constructed *Salmonella* Gallinarum ghost appears to be a promising vaccine candidate and can be used as a safe and highly immunogenic vaccine against FT although further safety and efficacy trials on the field are needed.

ively [45]. CD8⁺ T cells, also known as cytotoxic T cells, play an important role in immune protection against intracellular bacterial parasites such as *Listeria monocy-*

infections. Moreover, the role of sIgA or mu

The wells units (KEL),
mbH,

L of Flow cytometry analysis of CD3⁺, CD4⁺ and CD8⁺ subsets

T cell markers such as CD3⁺, CD4⁺, and CD8⁺ were

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Review

Development of a *Chlamydia trachomatis* bacterial ghost vaccine to fight human blindness

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Key words: vaccine, trachoma, bacterial ghost, *Chlamydia trachomatis*. immunology

Development of a *Chlamydia trachomatis* bacterial ghost vaccine to fight human blindness

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Key words: vaccine, trachoma, bacterial ghost, *Chlamydia trachomatis*, immunology

Trachoma is the world's leading cause of preventable disease and the third most common cause of blindness after cataract and glaucoma, affecting an estimated 84 million people and leaving 590 million at risk. As a crippling disease, trachoma causes an enormous loss of productivity and constitutes a major socio-economic burden. Although antibiotics are effective in treating active cases of the illness, they do not prevent re-infection, which occurs with high frequency in susceptible populations. Also, once infection and pathology are established, treatment may be less effective. Another major public health challenge posed by trachoma is that a large number of infected individuals are asymptomatic and readily infect those with whom they interact. Thus, an inexpensive and easy to deliver vaccine for trachoma would be highly effective in reducing the devastation caused by this disease. Development of an effective vaccine for controlling and preventing trachoma will require an understanding of the complex immunological mechanisms that occur during infection, identifying those antigens that elicit a protective immune response and designing effective vaccine delivery systems. Significant progress has been made in the delineation of the immune correlates of protection that will form the basis of vaccine evaluation. Recent advances in chlamydial genomics and proteomics has identified a number of protective antigens or epitopes that when appropriately delivered will produce an efficacious vaccine. The challenge at this time is the development of effective methods for vaccine delivery. We have developed an effective bacterial ghost (BG) delivery system possessing immunogenic

Introduction

Chlamydia trachomatis is an obligate intracellular Gram negative-like bacterium, composed of 15 odd serovars designated A through K and L1 to L3, which are distinguished by the antigenic variation in the major outer membrane protein.^{1,2} Serovars A, B, Ba and C cause ocular infections that can lead to trachoma. Serovars D through K and the lymphogranuloma venereum strains L1–L3 cause sexually transmitted diseases (STDs), primarily cervicitis and urethritis and that can lead to reactive arthritis. Pelvic inflammatory disease (PID) and tubal factor infertility (TFI) are major complications of genital *Chlamydia* infection and constitute an enormous morbidity and socioeconomic burden.³

Trachoma is the world's most common preventable blinding disease, affecting an estimated 84 million people and leaving 590 million at risk, with 8 million irreversibly blinded or severely visually impaired.⁴ The disease occurs particularly in many underprivileged communities with cluster living without adequate access to water and sanitation, reaching the poorest and rural areas in Africa, Middle-East, Asia, Latin America and Australia.⁵ In total 55 countries are endemic for trachoma. Children are the most susceptible to infection, but the signs and symptoms are often not felt until adulthood. Children build up a human reservoir for the spread of *Chlamydia trachomatis* and are the main source for reinfection in adults, predominantly the female caregivers, leaving women three times more at risk for blindness than men.⁶ Without intervention, trachoma leads to long-term disability, leaving families trapped within a cycle of poverty

nic high levels of HIV-1 reverse transcriptase. However, the immunogenicity of this recombinant construct was not evaluated. The possibility that *Chlamydia* may harbor pathologic components and vaccines are showing promise in inducing mucosal immunity in the mouse genital chlamydial infection model. A multisubunit vaccine consisting of VCG co-expressing multiple chlamydial outer membrane proteins induced higher frequency of Th1 cells and relatively greater ability to confer protective immunity compared to single subunit constructs.⁶²⁻⁶⁴ A major advantage of the multiple subunit approach is the potential synergistic immunologic benefit of a combination of epitopes from multiple antigens, which will likely induce a higher frequency of immune effectors that ensures an effective long-lasting immunity. In addition to their delivery capacity, VCGs provide adjuvant functions to the foreign delivered antigens as well. Intramuscular immunization of mice with VCG expressing the chlamydial major outer membrane protein (MOMP) induced significant local mucosal and systemic Th1 type responses. Notably,



The end

Thank you